The measurement of urine pH to predict the amount of buffer used in the treatment of acute rumen lactic acidosis in cattle

Mensuração do pH de urina para predizer a quantidade de tampão empregado para o tratamento de acidose láctica ruminal aguda em bovinos

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ABSTRACT

The purpose of the present study was to establish a practical, fast, precise and low-cost procedure to estimate the degree of metabolic acidosis in cattle with acute rumen lactic acidosis for further treatment. The rumen acidosis was induced experimentally in 40 crossbreed rumen-cannulated 1.5-yearold steers. The induction caused the development of the most characteristic clinical signs of acute rumen lactic acidosis, severe rumen acidosis and a moderate metabolic acidosis, which was evidenced by low blood pH, and blood bicarbonate concentration and base excess (BE). A highly positive correlation (r=0.80) between urinary pH and BE concentration, and between urinary pH and blood pH (r=0.75) was observed. The BE concentration estimated by urinary pH was similar to that determined by venous blood gas analysis (P>0.99). Furthermore, the results presented by the predictive formula were very significant. In conclusion, urinary pH is a good tool to predict the quantity of buffers needed to treat metabolic acidosis in cattle with acute rumen lactic acidosis.

Key words: lactic acidosis, urine pH, cattle, base excess, treatment.

RESUMO

O presente estudo teve como objetivo desenvolver um procedimento de baixo custo, preciso, rápido e prático para estimar o grau de acidose metabólica, para tratar bovinos com quadros de acidose láctica ruminal. A acidose ruminal foi induzida experimentalmente em 40 novilhos mestiços de 1,5 anos de idade, implantados com cânula ruminal. Essa indução causou o surgimento de sinais clínicos muito típicos da enfermidade aguda, com o aparecimento de pronunciada acidose ruminal e acidose metabólica de grau moderado, caracterizado por baixo pH sangüíneo e diminutos teores de bicarbonato e excesso de base (BE) no sangue. Verificou-se

uma alta correlação positiva (r = 0,80) entre o pH urinário e o BE e entre o pH urinário e o pH sangüíneo (r = 0,75). A concentração de BE estimado pelo pH urinário foi similar à obtida pela análise do hemogasômetro (P = 0,99). Além disso, os resultados apresentados pela fórmula de predição foram muito significativos. Dessa forma, conclui-se que a mensuração do pH urinário é uma boa alternativa para estimar a quantidade necessária de tampão para tratar o quadro de acidose metabólica em bovinos com acidose láctica ruminal aguda.

Palavras-chave: acidose láctica, pH urinário, bovinos, excesso de base, tratamento.

INTRODUCTION

The ingestion of large amounts of carbohydrate-rich feeds is the main cause of acute rumen lactic acidosis in cattle, mostly in non-adapted animals to this diet. This metabolic disease frequently occurs in intensive cattle management, mainly in feedlot beef cattle and dairy herds fed with high-level grain diets, affecting up to 50% of the herd. The mortality rate may be up to 90% in untreated cases, while 30-40% of treated cattle may die (RADOSTITS et al., 2002).

Acute rumen lactic acidosis occurs when there is an intense soluble carbohydrates fermentation in the rumen, which produces large amounts of lactic acid and provokes a drastic decrease in the rumen pH, characterizing rumen acidosis. Also, it elevates the osmolality of the rumen content above blood osmolality,

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which leads to dehydration due to fluid mobilization. A small amount of lactic acid may be absorbed rapidly into the blood stream and buffered by the plasma bicarbonate buffering system, which results in a drastic decrease in the blood pH and consequently metabolic acidosis, that could lead to death (DUNLOP, 1972).

Besides other treatments, such removal of the ruminal content and restoration of blood fluid and electrolytes, the correction of the systemic metabolic acidosis is essential for a successful recovery of the affected cattle (NETTO & ORTOLANI, 2000). In order to perform this treatment is necessary to verify the metabolic acidosis degree for determining the amount of buffer needed. It can be calculated by a traditional formula presented by CARLSON (1997): y = body weight (kg) x 0.3 x base excess (mmol L-1), where "y" represents the amount of bicarbonate (mmol) required to perform the treatment and "0.3" is a constant representing extracellular fluid volume. The base excess (BE) can be easily determined in a Blood Gas Analyzer, but this kind of equipment is expensive and is not always available under field conditions.

Thus, the correction of metabolic acidosis is in most cases empiric and imprecise, which may not correct the acidosis adequately; or, on the other hand, can result in a metabolic alkalosis due to excessive use of buffers, which is very difficult to reverse (HARTSFIELD et al., 1981).

HOWARD (1981) suggested to estimate the level of BE in cattle with rumen lactic acidosis through the degree of dehydration, being higher in the most dehydrated animals. However, a later study indicated that the correlation between dehydration and metabolic acidosis is low and the BE estimated is often under or overestimated when compared to the venous results obtained with a Blood Gas Analyzer (ORTOLANI et al., 1997). Thus it is still necessary to establish a practical, fast, precise and inexpensive method to estimate BE in cattle under field conditions.

During the metabolic acidosis the kidneys play an important role in the excretion of H⁺ ions, lowering the urine pH. This fact can reflect the blood pH (CARLSON, 1997). The present study aimed to evaluate the use of urine pH for determining the acidbase status and calculate the amount of buffer needed in the treatment of experimentally-induced cattle with acute rumen lactic acidosis.

MATERIAL AND METHODS

Forty rumen-cannulated crossbreed healthy yearling steers (250kg) were housed indoors in individual tie stalls and were fed with a basal diet

composed by 70% coast-cross hay (Cynodon dactylon (L) Pers) and 30% commercial concentrate. The total amount of dry matter was equivalent to 2.7% of their respective body weight for at least 40 days. This experiment protocol agreed with the Ethical Principles in Animal Research adopted by the local Bioethic Commission. Acute rumen lactic acidosis was experimentally induced through intraruminal administration of sucrose in order to obtain a rumen pH between 4.2 and 3.9, 20h after the induction, following a technique described previously (ORTOLANI, 1995). Samples of rumen fluid, urine and jugular blood were collected just before the induction and at the 20th and 24th h later. Thereafter all cattle were treated properly as follows: the rumen fluid was removed and replaced by three liters of rumen fluid from healthy cattle and seven liters of saline solution, beyond different amounts of intravenous infusion of sodium bicarbonate solution (1.3%) according to the formula proposed by CARLSON (1997), after the actual determination of BE.

The jugular blood samples were collected and analyzed according to LISBOA et al. (2001) to determine pH, bicarbonate, BE, and pCO₂^a. Urine samples were obtained through preputial massage, and rumen fluid was obtained by aspiration through rumen canula. Rumen fluid and urine pH were determined with a digital pHmeter.^b Total lactic acid was also determined in the rumen fluid and blood samples according to technique described by PRYCE (1969). The estimated BE, used to correlate with the real BE, was calculated by a regression equation, obtained between the correlation of urine pH and BE.

Data were analyzed with MINITAB (2000) statistical software using Student's *t* test for paired comparison. To determine if one variable is a predictor of another variable, it was used simple linear regression analysis and calculated the Pearson sample correlation coefficient, with significance confirmed by the *F* test (SAMPAIO, 1998). To evaluate use of urine pH to find out the acid-base status, it was determined the sensitivity, specificity, positive and negative predictive value, and accuracy (MONFORT & MILLER, 1990). All cattle used in this experiment had rumen pH lower than 4.5 at the 20th h and typical clinical signs of acute rumen lactic acidosis, such as tachycardia, reduced number of rumen movements, anorexia and diarrhea.

A steer was considered positive for metabolic acidosis and in the prediction test if the blood pH and BE concentration were below to the respective cut-off value (7.29 for pH and -2.3mmol L^{-1} for BE) according to ORTOLANI (2003b). Results obtained at the 20^{th} and 24^{th} h equal or above cut-off values were

considered negative for metabolic acidosis in the prediction test.

RESULTS

All the variable measured had similar values at the 20^{th} and 24^{th} h (P < 0.01). On the 20^{th} h after dosing sucrose, the rumen fluid pH had decreased from 6.94 ± 0.23 to 4.30 ± 0.1 (P < 0.004) and mean concentration for total lactic acid in the rumen fluid increased from 0.3 ± 0.1 to 116 ± 10 mmol L⁻¹ and in the blood from $1.2\pm$ $0.3 \text{ to } 8.4 \pm 2.6 \text{mmol L}^{-1}$ (P < 0.0001). Higher mean values (P < 0.0001) of blood pH, bicarbonate and BE and urinary pH were obtained before (7.37±0.05; 25.3±2.8mmol L⁻¹ and 1.0±2.9mmol L⁻¹; 7.21±0.79, respectively) than after dosing with sucrose (7.22±0.05; 17.6±3.3mmol L⁻¹ e -8.9±3.9mmol L⁻¹; 5.49±0.64, respectively). The paired analysis of the urinary pH difference between the moment before dosing sucrose and the 24th h indicated a decrease of 2.1±0.9 pH units.

The results showed reduction in the blood pH and in the urinary pH (r=0.75; P < 0.0001; Figure 1). Similar pattern was observed between urinary pH and BE levels (r = 0.80; P < 0.0001; Figure 2). There was no significant difference (P > 0.99) in overall results between the mean values of BE estimated by urinary pH (-6.47±4.45) and BE values determined by blood gas analysis (-6.47±5.60).

The performance of the prediction tests of blood pH and BE values through the use of urinary pH are shown in figures 3 and 4, respectively. The results

for blood pH prediction were: sensitivity 95.0%, specificity 69.0%, positive predictive value 87.4% and negative predictive value 85.7%; and the results for BE prediction were: sensitivity 96.5%, specificity 79.3%, positive predictive value 93.3% and negative predictive value 88.5%.

DISCUSSION

The sucrose used in the present study with sucrose successfully produced rumen lactic acidosis, which could be verified by a marked elevation in the rumen total lactic acid concentration that led to a drastic fall in the rumen fluid pH. Some of this lactic acid was absorbed into the bloodstream, which resulted in several clinical implications such as apathy, tachycardia, different degrees of dehydration, reduced rumen movements, anorexia, diarrhea, in many cattle oliguria, recumbency, reluctance to move and muscular weakness. Thus, after the 20th h there were a decrease in the mean values of blood pH (7.21), bicarbonate (17.6mmol L⁻¹) and BE (-8.9mmol L⁻¹). These values characterized a moderate metabolic acidosis according to RADOSTITS et al. (2002), requiring a proper treatment at the end of the experiment. Some cattle had BE values as low as – 17mmol L⁻¹ and exhibited the most severe clinical signs such as recumbency, reluctance to move and mental depression, requiring an urgent treatment. Nevertheless, the adopted treatment was very efficient to correct the rumen acidosis and mainly to restore the blood pH in all cases.

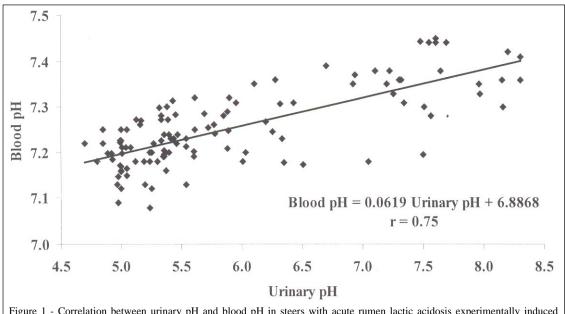


Figure 1 - Correlation between urinary pH and blood pH in steers with acute rumen lactic acidosis experimentally induced with sucrose (n = 120).

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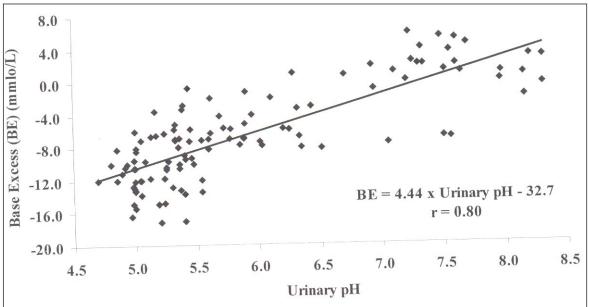


Figure 2 - Correlation between urinary pH and blood BE in steers with acute rumen lactic acidosis experimentally induced with sucrose (n = 120).

The decrease in the blood pH was followed by a concomitant fall (r=0.75) in the urinary pH (Figure 2). The kidneys play a very important role in the correction of the acid-base balance during the metabolic acidosis (ORTOLANI, 2003a). At the same time the proximal convoluted tubules are able to excrete H⁺ and to produce and reabsorb adequately bicarbonate into the bloodstream (CARLSON, 1997; DUNLOP, 1972). In fifteen percent of the animals, the urine samples obtained from the 20th and 24th h had a urinary pH lower than 5.0. In one particular case the urine pH reached 4.7 at the 24th h lowering 2.6 units as compared to value of the beginning of the experiment (pH 7.3). Since the

pH is expressed in a logarithmic scale, it means a 400-fold increase in the $H^{\scriptscriptstyle +}$ excretion in the urine. Although the mean difference between the urinary pH of all animals from the $24^{\rm th}$ h to the beginning was lower (2.1 pH units), there was a considerable 130-fold increase in the $H^{\scriptscriptstyle +}$ excretion in the urine.

There was no significant difference (P>0.99) between the BE estimated by the formula obtained from the regression analysis and the actual BE measured by the Blood Gas Analyzer. The significant results of sensitivity (96.7%), positive predictive value (93.6%) and accuracy (92.5%) highly validated the use of the present formula [BE (mmol L^{-1}) = 4.44 x Urinary pH –

		Rumen lactic acidosis			Sensibility	= 95.3%
		Positive	Negative		Specificity ,	= 68.6%
T e s t	Positive	81	11	92	Positive predictive value	= 88.0%
	Negative	04	24	28	Negative predictive value	= 85.7%
		85	35	120	Accuracy	= 87.5%

Figure 3 - Performance of the prediction formula of blood pH (Blood pH = 0.0619 x Urinary pH + 6.8868) using urinary pH from steers with acute rumen lactic acidosis experimentally induced with sucrose.

		Rumen lactic acidosis			Sensibility	= 96.7%
		Positive	Negative		Specificity	= 79.3%
T e s t	Positive	88	06	94	Positive predictive value	= 93.6%
	Negative	03	23	26	Negative predictive value	= 88.5%
		91	29	120	Accuracy	= 92.5%

Figure 4 - Performance of the prediction formula of blood BE (BE = 4.44 x Urinary pH - 32.7) using urinary pH from steers with acute rumen lactic acidosis experimentally induced with sucrose.

32.7] to predict the value of BE. Thus, is possible to calculate the amount of needed buffer for correcting the metabolic acidosis due to acute lactic acidosis in cattle.

In few cases false negative results were obtained by the use of urinary pH to predict blood pH (4.7%) or BE concentration (3.3%). The urinary pH could be elevated in some cases of metabolic acidosis when there is a deficiency in the bicarbonate absorption by the nephrons. It may occur when there is a respiratory acidosis (due to some respiratory disease) and an increase in the blood pCO_2 , which negatively interferes in the bicarbonate absorption by the proximal convoluted tubules, resulting in its excretion in the urine and rising the urinary pH (ORTOLANI, 2003a). By coincidence two acidotic steers had high urinary pH (7.05 and 7.50) and high pCO_3 values (57.4 and 56.7mmHg, respectively; reference values: 35-44mmHg; CARLSON, 1997), showing that they may have had a mild respiratory acidosis. Normally, in the acute rumen lactic acidosis there is no significant change in the pCO₂ (ANGELOV et al., 1995), so those two cases of respiratory acidosis were exceptions. Another possibility to explain the presence of a false negative in a single steer could be the interference of residual urine produced before the development of metabolic acidosis increasing the urine pH thereafter. Nevertheless, the sensibility was very high (96.7 %) indicating that this was an isolated case.

Although the specificity obtained was high (79.3%) (Figure 4), in 6 cases the formula did not correctly estimated the BE value in animals, which that did not have acute rumen lactic acidosis, resulting in 6.4% of false positives. In other words, the formula

indicated the unnecessary use of treatment for correction of metabolic acidosis when the animals did not need it. Nevertheless, the amounts of bicarbonate infusion in these cattle were small and no collateral effects were seen at all. A more detailed study of these six cases indicated that to result in false positives the urinary pH should be between 6.7 and 5.5, which are within reference values for bovines (pH 5.5-8.0; ORTOLANI, 2003a) fed with concentrate and forage. These results indicate that the present prediction formula for treating metabolic acidosis must be used only in animals with definite clinical signs of acute rumen lactic acidosis. Thus, if this recommendation is followed correctly the number of cases of false positives will be confined to a minimum.

A similar research showed that urinary pH could be used to predict blood pH and BE in calves with metabolic acidosis due to diarrhea, with higher correlation between the variables (0.77 and 0.72, respectively) (LUBETSKAYA & MELNICHUK, 1999), demonstrating coherence of the concepts presented by the present study. However, the formula originated by the present study must not be used in calves with diarrhea because the normal urinary pH of calves is higher (6.2-7.3), and its cut-off value for calves with metabolic acidosis is below 6.0, whereas in adult cattle with rumen lactic acidosis is below 5.5.

CONCLUSION

The present research demonstrated that urinary pH can be used, with high accuracy, to predict the base excess values in cattle with experimentally acute rumen lactic acidosis. The presented formula can 722 Maruta et al.

be used to estimate the amount of buffer needed to correct the metabolic acidosis, and avoid the use of an expensive and complex analysis of base excess in a Blood Gas Analyzer.

SOURCE

^aBlood Gas Analyzer Radiometer[®], model ABL 330 – Copenhagem: Dinamarca. ^bpHmeter Celm[®], model pH-10- Baueri: São Paulo.

ACKNOWLEDGMENTS

The technical support of Regina Mieko Sakata Mirandola is gratefully acknowledged. Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) supported this research with a scholarship.

APROVED BY THE BIOETHICS COMMITTEE

USP 1123/2007

REFERENCES

ANGELOV, G. et al. A comparative study on acid-base status in ruminants with experimental rumen acidosis. **Veterinarski Arhiv**, v.65, p.135-141, 1995.

CARLSON, G.P. Fluid, electrolyte, and acid-base balance. In: KANEKO et al. **Clinical biochemistry of domestic animals**. 5.ed. San Diego: Academic, 1997. p.485-516.

DUNLOP, R.H. Pathogenesis of ruminant lactic acidosis. Advances in Veterinary Science and Comparative Medicine. v.16, p.259-302, 1972

HARTSFIELD, S.M et al. Sodium bicarbonate and bicarbonate precursors for treatment of metabolic acidosis. **Journal of the American Veterinary Medical Association**, v.179, p.914-916, 1981.

HOWARD, J.L. Ruminal metabolic acidosis. **Bovine Practitioner**, v.1, p.44-53, 1981.

LISBOA, J.A. et al. Viability time of blood gas analysis in bovine venous blood samples stored in ice water bath. **Ciência Rural**, v.31, p.271-276, 2001.

LUBETSKAYA, T.; MELNICHUK, D. Urine pH as an index for calculating the amount of bicarbonate for treatment of

acidotic calves. **Journal of Animal and Feed Sciences**, v.8, p.247-254, 1999.

MINITAB-The student edition of MINITAB Statistical adapted for education-13.0 release. **User'r Manual**. New York: Addison_Wesley, 2000. 120p.

MONFORT, J.D.; MILLER, G.Y. Evaluation and application of diagnostic tests in food animal practice. **Compendium on Continuing Education for the Practicing Veterinarian**, v.12, p.1182-1190, 1990.

NETTO, D.M.; ORTOLANI, E.L. Evaluation of sodium bicarbonate or lactated ringer's solution for the treatment of rumen lactic acidosis in steers. **Veterinária Notícias**, v.6, p.31-39, 2000.

ORTOLANI, E.L. Induction of lactic acidosis in cattle with sucrose: Relationship between dose, rumen fluid pH and animal size. **Veterinary and Human Toxicology**, v.37, p.462-464, 1995.

ORTOLANI, E.L et al. O uso do pH urinário para estimar o grau de acidose metabólica em garrotes com acidose lática ruminal. In: CONGRESSO BRASILEIRO DE MEDICINA VETERINÁRIA, 25., 1997, Gramado, RS. Anais... Gramado: SOVERGS, 1997. p.215.

ORTOLANI, E.L. Diagnostico de doenças nutricionais e metabólicas por meio de exame de urina em ruminantes. In: SIMPOSIO DE PATOLOGIA CLINICA VETERINARIA DA REGIAO SUL DO BRASIL, 2003a, Porto Alegre, RS. Anais... Porto Alegre: UFRGS, 2003. p.91-102.

ORTOLANI, E.L. Diagnostico e tratamento de alterações ácido-básicas em ruminantes. In: SIMPOSIO DE PATOLOGIA CLINICA VETERINARIA DA REGIAO SUL DO BRASIL, 2003b, Porto Alegre, RS. **Anais...** Porto Alegre: UFRGS, 2003. p.17-29.

PRYCE, J.D. A modification of the Barker-Summerson method for the determination of lactic acid. **Analyst**, v.94, p.1151-1152, 1969.

RADOSTITS, O.M. et al. **Clínica veterinária: um tratado de doenças dos bovinos, ovinos, suínos, caprinos e eqüinos.** 9.ed. Rio de Janeiro: Guanabara Koogan, 2002. 1737p.

SAMPAIO, I.B.M. Estatística aplicada à experimentação animal. Belo Horizonte: Fundação de Ensino em medicina vaterinária e zootecnia, 1998. 221p.